Glycoprotein V: the predominant target antigen in gold-induced autoimmune thrombocytopenia

Stephen F. Garner, Kate Campbell, Paul Metcalfe, Jane Keidan, Elly Huiskes, Jing-Fei Dong, José A. López, and Willem H. Ouwehand

Autoimmune thrombocytopenia (AITP) in patients with rheumatoid arthritis (RA) on gold therapy is a rare, but severe, event. Platelet autoantibodies generally target glycoprotein (GP) IIb-IIIa or GPib-IX, and occasionally against GPIa-IIa or GPV. By investigating 38 patients on gold therapy, 10 with profound thrombocytopenia and 28 non-thrombocytopenic controls, we showed that in all patients with thrombocytopenia, the platelet autoantibodies preferentially targeted GPV but the presence of gold was not required for their reactivity. Elevated levels of platelet-associated IgG (PAIgG) were observed in 8 of the 10 patients in whom the tests were performed. In 5 patients with sufficient autologous platelets, the GPV specificity of PAIgG was confirmed. Tests with GPV transfectants revealed that the antibodies reacted with GPV independent of GPIIb, GPIb\(\alpha\), or GPIX. Autoantibodies recognizing GPV were not seen in the 28 nonthrombocytopenic control RA patients. Thus, GPV seems to be targeted in gold-induced autoimmune thrombocytopenia. (Blood. 2002;100:344-346)

Introduction

Autoimmune thrombocytopenia (AITP) in patients with rheumatoid arthritis (RA) on gold therapy is a rare, but severe, event. Platelet autoantibodies generally target glycoprotein (GP) IIb-IIIa or GPib-IX, but GPV seems to be targeted in gold-induced autoimmune thrombocytopenia. For direct immunofluorescence, the patients' platelets were washed using phosphate-buffered saline containing 0.01 M EDTA and 0.25% bovine serum albumin. Fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human IgG (1 µg per 5 × 10⁶ platelets) was then added, and after 30 minutes at 20°C the platelets were washed again and examined for bound FITC by flow cytometry. The patients' own platelets were also used for the direct MAIPA as follows. After washing, platelets were incubated with these GPV-specific monoclonal antibodies (mAbs), used as either culture supernatants (1 in 10 dilution) or ascites (1 in 1000 dilution): RFGP56 for GPIIb-IIIa, CLB-MB45 for GPIb-IX, CLB-SW16 for GPV, and CLB-10G11 for GPIa-IIa. Following further washes, the platelets were solubilized and the lysates were added to microplate wells coated with goat anti-mouse Ig to capture the mAb-GP complexes. Bound human antibodies were then revealed with goat anti-human Ig. For the indirect MAIPA, sera were tested against platelets of known human platelet antigen (HPA) genotype. Results from all assays were compared with those obtained with platelets or sera from healthy blood donors tested in parallel.

Purified IgG fractions were prepared from 5 patients' sera using Mab Trap GII kits (Amersham Pharmacia Biotech, Bucks, England), according to the manufacturer’s instructions. The sera and IgG fractions were retested in MAIPA in the absence and presence of gold at final concentrations of 0.1 to 100 µg/mL.

In 4 patients with thrombocytopenia there were sufficient sera for a sandwich enzyme-linked immunosorbent assay (ELISA) with a mouse fibroblast L-cell line stably expressing human GPV. A 3.5-kilobase (kb) DNA fragment encoding GPV was cloned into the ZEM229R expression vector and transfected as described previously. The assay was based on the indirect MAIPA, with the L cells incubated with the patients' sera and...
GPV-specific mAb before solubilization and subsequent capture of the mAb-GP complexes. Use of these transfectants enabled further definition of antibody specificity because, unlike platelets, GPV in these cells is expressed without the associated GPIbα, GPIbβ, and GPIX.

**Results and discussion**

Increased PAIgG levels were detected in all 8 AITP patients tested, with results between 1.9 and 22.8 times higher than in the negative controls (Table 1). Direct MAIPA in 5 patients tested showed almost exclusive GPV reactivity, with the ELISA OD values clearly greater than those obtained with controls (Table 1). Reactivity with capture mAbs against other GPs was negative in all but one patient (patient 7) in whom weak reactivity with GPIIb-IIIa was seen. The average OD for the 10 sera was 0.07 (SD = 0.06), and the negative control sera gave an average OD of 0.07 (SD = 0.07). Similarly, reactivity was not seen with GPIIb-IIIa or with GPIa-IIa.

GPV-specific antibodies were detectable in purified IgG from all 5 patients tested (OD values 0.34 or greater; Table 2). Addition of gold to the sera and purified IgG at concentrations ranging from 0.1 to 100 µg/mL did not enhance reactivity (Table 2). These results indicate that gold does not act as a hapten. It therefore appears to break tolerance for self by inducing autoantibody formation, as is found with α methylidopa-induced autoimmune hemolytic anaemia22 rather than by mechanisms of drug adsorption or by the formation of trimolecular complexes of drug, antibody, and cell membrane.

That gold is not required for autoantibody binding agrees with earlier studies using eluted autoantibodies.23 However, our specificity results are not in agreement with a previous suggestion that autoantibodies in gold-induced AITP are against GPIIb-IIIa.23 This latter conclusion was inferred from the lack of reactivity with platelets from patients with Glanzmann thrombasthenia rather than from positive test results.

To investigate whether the epitopes recognized by autoantibodies are uniquely expressed on GPV, sera from patients 6, 7, 8, and 9 were tested using ELISA with fibroblasts expressing human GPV.

**Table 1. Results from patients with thrombocytopenia**

<table>
<thead>
<tr>
<th>Case</th>
<th>Platelet count (&lt; 10⁵/L)</th>
<th>Direct PIFT T/C ratio*</th>
<th>OD values</th>
<th>Direct MAIPA†</th>
<th>Indirect MAIPA‡</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>Patient</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3.6</td>
<td>0.13</td>
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<tr>
<td>2</td>
<td>6</td>
<td>6.8</td>
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<td>0.23</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
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<td>nt</td>
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<tr>
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<td>nt</td>
<td>0.01</td>
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<tr>
<td>5</td>
<td>35</td>
<td>8.8</td>
<td>0.01</td>
<td>1.63</td>
<td>0.02</td>
</tr>
<tr>
<td>6</td>
<td>58</td>
<td>3.9</td>
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</tr>
<tr>
<td>7</td>
<td>60</td>
<td>1.9</td>
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<td>&gt; 3.5</td>
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<tr>
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<td>nt</td>
<td>nt</td>
<td>0.23</td>
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<tr>
<td>9</td>
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<td>nt</td>
<td>0.02</td>
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<tr>
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<td>na</td>
<td>na</td>
<td>nt</td>
<td>nt</td>
<td>0.09</td>
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</table>

‡ELISA OD values. Results are from a representative experiment, derived from duplicate testing against platelets from 2 donors.

†Negative control AB serum or IgG fraction from AB serum.

§Result not above cut-off of twice the control result.

#Autoantibodies were eluted from the patient’s platelets and tested by MAIPA.

‡ELISA OD values. Results are from a representative experiment, derived from duplicate testing against platelets from 2 donors.

Antibodies to GPIb-IX were not seen. The average OD for the 10 sera was 0.07 (SD = 0.06), and the negative control sera gave an average OD of 0.07 (SD = 0.07). Similarly, reactivity was not seen with GPIIb-IIIa or with GPIa-IIa.

**Table 2. Indirect MAIPA with serum and IgG fractions, in the absence and presence of gold**

<table>
<thead>
<tr>
<th>Case</th>
<th>Native sample</th>
<th>Gold (µg/mL)*</th>
<th>Purified IgG</th>
<th>Native sample</th>
<th>Gold (µg/mL)</th>
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<td>8</td>
<td>1.12</td>
<td>0.95</td>
<td>1.02</td>
<td>1.45</td>
</tr>
</tbody>
</table>

*Final concentration of gold during all stages of MAIPA. For clarity, results using 100 µg/mL are not shown.

†ELISA OD serum or IgG fraction from AB serum.

‡ELISA OD values. Results are from a representative experiment, derived from duplicate testing against platelets from 2 donors.

Native samples are samples without gold added.

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All sera gave positive results, and OD values were 0.31, 0.27, 0.25, and 0.37, respectively, compared with a value of 0.03 with the negative control. This reactivity demonstrates that the epitopes are confined to GPV and are not dependent on the presence of GPIba, GPIbβ, or GPIX.

All but one of the 28 negative control RA patients without thrombocytopenia (platelet counts 153 × 10^9/L or higher; range, 153-582 × 10^9/L; mean, 285 × 10^9/L) had a negative direct PAIgG test. The single positive patient had a platelet count of 329 × 10^9/L, and the PAIgG result was 2.9 times that of the negative control. There was insufficient sample for further testing of the PAIgG, but indirect testing showed a weak alloantibody of undetermined specificity.

In summary, we obtained significant evidence that in gold-associated AITP, autoantibodies recognize GPV. Cloning of these autoantibodies is required to determine whether the loss of tolerance is for single or multiple epitopes and whether the same epitopes are targeted in the different clinical groups. Such recombinant mAbs would also be ideal tools for investigating the functional effects of GPV-specific antibodies because it has recently been suggested that they may affect collagen-induced platelet aggregation.24

**Acknowledgments**

We thank Professor A. E. G. Kr von dem Borne and Dr L. Porcelijn for their gifts of the CLB mAbs and for referring patient 10, respectively. We also thank Dr Peter Smethurst for the IgG purification, the staff at the Platelet Immunology Reference Laboratory at the National Blood Service East Anglia Centre for their expertise in serology, and Dr John Williams for reviewing the rheumatoid arthritis cases.

**References**

7. Proulle V, Masnou P, Cartron J, et al. GPIaIIa antibodies because it has recently been suggested that they may affect collagen-induced platelet aggregation.
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